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Biologically active compounds of *Prunus spinosa* L. mature fruits and prospects for their use

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The article presents the results of determining the nutritional and biologically active components of the ripe fruits of prickly plum (*Prunus spinosa* L.), growing in Azerbaijan. It Magiera has been established that the fruits of the plant are rich in various nutritional and biologically active components, such as sugars, organic acids, pectin and tannins, flavonoids, anthocyanins and vitamin C. From simple sugars, fructose (2.82%), glucose (1.81%), sucrose (0.81%) and xylose (0.18%), citric, malic and succinic acids were identified in the composition of organic acids. In addition, the content of pectin were 2.3% and vitamin-C were 230 mg. The method of two-dimensional chromatography revealed 3 anthocyanidins in the sum of anthocyanins, identified as cyanidin-3-glucoside (chrysanthemin) and cyanidin-3,5-O-diglucoside (cyanine). Two individual substances were isolated by preparative paper chromatography, identified as quercitrin and rutin.
Keywords: Prickly plum, nutrition, biologically active components, chromatography, Azerbaijan.

INTRODUCTION

The flora of Azerbaijan is rich in various useful plants. Among them, wild-growing fruit and berry plants are of particular interest, which contain many biologically active substances in various organs, and especially in fruits (Ibadullayeva and Husneynova, 2021). Such plants include prickly plum (*Prunus spinosa* L.) from the Rosaceae family (*Rosaceae* Adans (Juss.). This species is widely distributed in all botanical-geographical regions of Azerbaijan, as well as in the Caucasus, Central Asia, and Europe (Verkhozina *et al*., 2021).

Prickly plum (sometimes called blackthorn in the literature) is an excellent food, medicinal, ornamental, melliferous, perganous plant (Bahrin *et al*., 2022). Mature fruits of the plant are widely used by the population for making jam, marmalade, compotes, kvass, vinegar and marinades while in France its marinade from unripe fruits is used as a substitute for olives (Trabelsi *et al*., 2021).

Various organs of prickly plum have long been used in folk and scientific medicine. A decoction and infusion of leaves and flowers has a diuretic effect in diseases of the kidneys, prostate, plant roots used in gastrointestinal diseases. Fruit juice has antibacterial effect. The fruits have cleansing abilities and remove harmful components of toxic substances from the body, and also normalize the intestinal microflora (Magiera *et al*., 2022). Decoctions from the leaves, flowers and branches of the plant are very valuable for liver diseases and metabolic disorders in the body (Benkhnigue *et al*., 2023). The fruits are actively used for weight loss, dizziness, nausea, increased irritability, to reduce harmful cholesterol in the blood (Opriş *et al*., 2021).

There is a lot of information in the literature about the chemical composition of fruits containing sugars, organic acids, various vitamins and provitamins (carotene), coumarins (umbelliferon, scopoletin), higher aliphatic hydrocarbons, etc. The presence of palmitic, stearic, palmitoleic, oleic, linoleic, eleostearic acids was also found in the oil from the kernel of the prickly plum (Sabtaini *et al*., 2020).

Leaves and flowers of the plant contain kaempferol, quercetin, hyperoside, rutin, triterpenes, and sterols (Veličković *et al*., 2020). Seventy substances were found in the leaves of the plant, of which 20 identified components belong to phenolcarboxylic acid, derivatives of oxystilbene, coumarin, flavonol, and isoflavonoid by high performance liquid chromatography (Macetic *et al*., 2022). In the leaves before and after flowering, and in the fruits of prickly plum,

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27 elements were found, including 4 macro (Na, K, Ca, P) and 24 microelements (for names see Table 2).

Analysis of literature data shows that there is still no accurate data on the chemical composition of fruits, especially the quantitative composition of carbohydrates, flavonoids, anthocyanins, organic acids of prickly plum growing in Azerbaijan (Blagojević *et al*., 2022). These substances are of interest both from a pharmacological and therapeutic point of view.

In this regard, the purpose of this work was to establish the component composition of carbohydrates, organic acids, flavonoids, anthocyanins of ripe prickly plum fruits growing in Azerbaijan.

MATERIALS AND METHODS

***Fruit Collection*:** The fruits of prickly plum were collected in the phase of biological maturity from the Goygol Reserve (now the Goygol National Park on a surface area of 12,755 hectares, among the shrub association consisting of *Rosa canina* (dog rose), *Sambucus nigra* (Elderberry), *Prunus divaricate* (cherry plum) and *Berberis vulgaris* (European barberry) *(I suggest to write English names also)* in August 2022. Around six hundred plum fruits were collected on arbitrary basis from naturally growing trees. Each time, sampling was executed from the trees which were selected at the beginning of the research, to ensure uniformity of the source plants throughout the research duration. Three trees were selected from every site. No anthropic action was conducted in the selected site of trees. Later on the collected fruits were brought to the lab, placed in refrigerated boxes and completely mixed prior to be grouping into well-mixed triplicates. Complete fruits comprising of central hard part, stone and outer pulpy part were freeze-dried at -80◦C in an Ultra-low temperature freezer (BIO-ULTRA UL570 A/S Denmark). Freeze-dried replicates were grounded into a fine powder, stored hermetically, protected from light, and kept the deep freezer at the mentioned temperature for proximate analyses (fruits were placed for fourteen days). Outcomes were shown in mg/100 g of fresh weight.

***Proximate analysis of Plum Fruits*:** Proximate analyses of Lyophilized plum fruits were carried out by the standard protocols defined by the Association of Official Analytical Chemists (AOAC, 2019).

***Dry matter determination*:** Fruit sample of 2.5 g was taken in the porcelain capsule, kept in an oven for 12 hrs at 105◦C, later on placed in desiccator to cool down the sample temperature and weighed for the dry weight (Brusewitz, 1975).

$$\text{Dry weight (\%)} = \frac{\text{Weight of the fruit sample after drying}}{\text{Initial weight}} \text{ x100}$$

***Sugar contents*:** The entire types of sugars were aproximated according to the methods as described by Horwitz (1960). Measure 25 ml of fruit juice was poured in 100 ml flask; 20 ml of distilled water and 5 ml of H_2SO_4 were too incorporated for transforming the sucrose, and other non-reducing sugars into reducing ones. Then neutralization of resulting material was executed with an alkali solution. Phenolphthalein was used for color indication. Later on the solution was poured into the burette and titration was done by 10 ml Fehling's solutions for determination of entire sugars contents.

***Crude protein analysis*:** From the fruit sample, total soluble solids (TSS) were calculated through a procedure illustrated by Dong *et al*. (2001). Single cut of uniform dimension was taken from 10 fruits and juice was extracted to turn it into a blend for every treatment in every duplication. After this, a hand refractometer (Abbe® Model 10450) was used to record the TSS from the prepared juice samples.

***Vitamin C content (mg/Kg)*:** This vitamin in the juice samples was assessed through 2, 6, dichlorophenol indophenol dye following a procedure as illustrated by Ruck (1963). Organic acids and pectin was determined according to Ermakova *et al*. (1972), ascorbic acid according to Sunthornsuk spellin (2002), anthocyanins and flavonoids according to Novruzov (2004), the qualitative composition of sugars and organic acids according to Soldatenk and Mazurov (1962).

RESULTS

The results of the chemical analyses of fresh fruits of prickly plum, collected in the phase of biological maturity, are presented in the Table 1.

Table 1. Chemical composition of fruits of prickly plum (*Prunus spinosa*).

Investigated Substances	Content % wet weight
Dry matter	29.31
Sum of sugars	5.44
Organic acids	4.01
Pectin	2.31
Tannins	0.94
Flavonoids	0.32
Anthocyanins	2.33
Vitamin C	0.23

From the data of Table, it can be seen that the fruits are rich in various nutrients and biologically active substances, such as sugars, organic acids, pectin and tannins, flavonoids, anthocyanins and vitamin C.

Table 2 indicates clearly that among the Macroelements in the type of raw materials under study, potassium (K) predominates, among the microelements-magnesium (Mg), silicon (Si), aluminum (Al), iron (Fe) and manganese (Mn). (May I suggest to write all miroelements in desending order to make them very clear, or in alphabetical order ??). After flowering in the leaves, the content of calcium (Ca), barium (Ba), strontium (Sr) decreases, the content of phosphorus (P),

silicon (Si), iron (Fe), aluminum (Al), zinc (Zn), molybdenum (Mo), boron (B), manganese (Mn), titanium (Ti), vanadium (V), chromium (Cr), zirconium (Zr) increases; the content of potassium (K), sodium (Na), copper (Cu), silver (Ag), nickel (Ni) and beryllium (Be) remains unchanged. Elemental composition of leaves and fruits of prickly plum, *Prunus spinosa* L. ~~he~~ emphasizes that the specie has a wide range of perspectives in the development of new medicines and cosmetic preparations.

Table 2. Mineral composition of leaves and fruits of prickly plum, *Prunus spinosa* (%ash content).

Sr.	Raw Material	Leaves (before flowering)	Leaves (after flowering)	Fruits
Macroelements				
1	Phosphorus (P)	3.001	5.010	5.010
2	Potassium (K)	30.001	30.001	30.001
3	Sodium (Na)	0.598	0.598	1.500
4	Calsium (Ca)	10.020	5.000	1.500
Microelements				
1	İron (Fe)	0.201	0.300	0.120
2	Magnesium (Mg)	2.032	5.000	3.000
3	Aluminum (Al)	0.302	0.505	0.100
4	Silica (Si)	2.065	3.023	0.300
5	Copper(Cu)	0.005	0.005	0.008
6	Zink (Zn)	0.011	0.030	0.006
7	Plumbum (Pb)	0.010	0.006	0.0006
8	Silver (Ag)	0.000011	0.000011	0.000015
9	Molybdenum (Mo)	0.0001	0.0003	0.0001
10	Barium (Ba)	0.056	0.029	0.029
11	Strontium (Sr)	0.067	0.059	0.039
12	Bor (B)	0.003	0.100	0.060
13	Manganese (Mn)	0.107	0.150	0.030
14	Nickel (Ni)	0.002	0.002	0.002
15	Titan (Ti)	0.025	0.030	0.010
16	Vanadium (V)	0.0003	0.0005	0.0001
17	Crome (Cr)	0.011	0.022	0.011
18	Zirkonium (Zr)	0.011	0.022	-
19	Beryllium (Be)	0.00015	0.00015	0.00015
20	Bismuth (Bi)	-	-	-
21	Tin (Sn)	-	0.0003	0.002
22	Gallium (Ga)	0.0001	0.0002	-
23	Lithium (Li)	-	-	0.001
24	Cobalt (Co)	-	0.0001	-
	Total	48.62%	49.89%	41.73%

Data on chemical and physical properties of the fruit is presented (Table 3) which revealed that the fruit length was 11.43 mm, width (12.92 mm), weight (2.31 g) while flesh/seed ratio was recorded as 3.95.

Table 3. Chemical and Physical characteristics of Pricky plums fruit.

Parameters	Mean ± SE
Length (mm)	11.43±1.52
Width (mm)	12.92±1.32
Weight (g)	2.31±0.76
Flesh/seed ratio	3.95±0.84

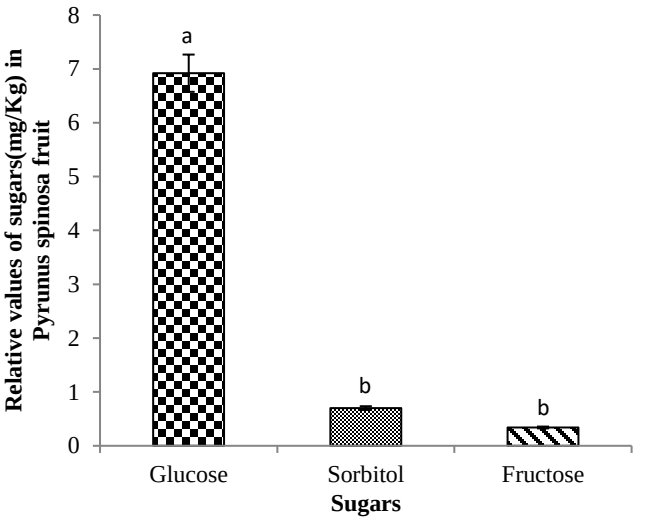

Figure 1. Reveals the realative values of different types of sugar groups in *Pyrunus spinosa* fruit samples. Vertical bars show standared error of mean values. Mean values sharing similar letter are statistically different.

The results (Fig. 1) showed variation in relative amounts of different sugars in pricky plum fruit sample. Glucose was recorded as highet (6.92 mg/Kg) while the values of Sorbitol and Fructose sugars were 0.70 mg/Kg and 0.34 mg/Kg of the fruit.

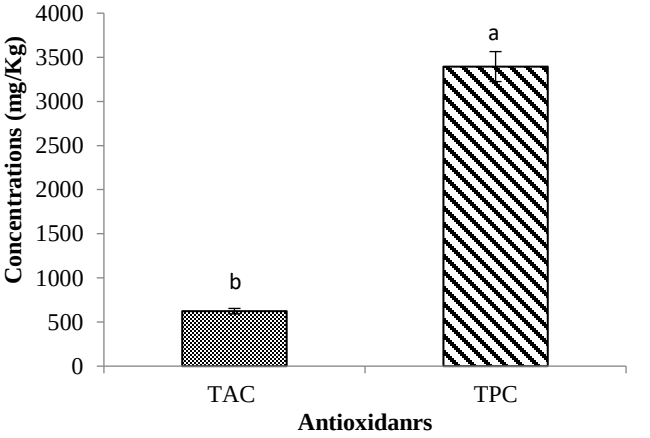

Figure 2. Realative values of different types of Antioxidants in *Pyrunus spinosa* fruit sample. Vertical bars show standared error of mean values. Mean values sharing different letters are **statistically** different. TPC= Total Phenolic Content, TAC=Total Anthocyanin Content.

The data in Fig. 2 revealed contents (mg/Kg) of different antioxidants in pricky plum fruits. Highest content (3394.61

mg/Kg) was recorded for TPC while low content (623.19 mg/Kg) was recorded in case of TAC

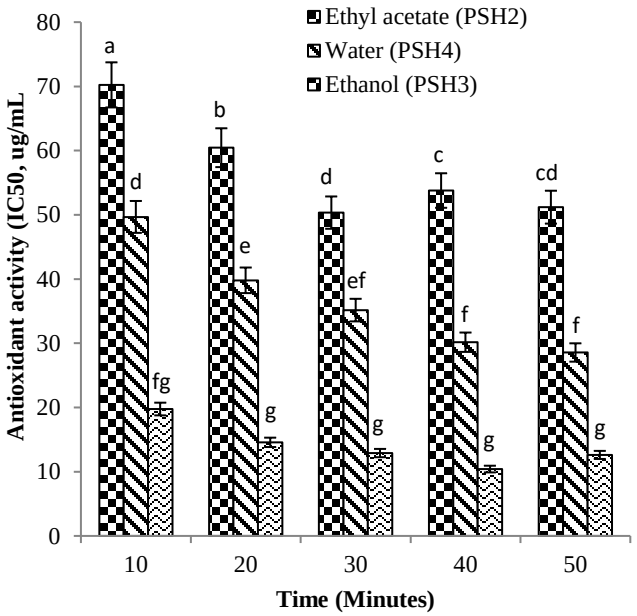

Figure 3. Presents Antioxidant activity (IC_{50}, µg/mL) of different extracts of Prunus spinosa leaves. Vertical bars show standared errors of mean values. Mean values sharing different letters are statistically different and Vice versa at (α=5%).

Results indicates that maximum antioxidant activity (70.23 IC_{50}, µg/mL) was recorded in case of Ethy acetate extract of *P. Spinosa* leaves followed by water extract (49.67 IC_{50}, µg/mL) while less activity (19.76 IC_{50}, µg/mL) was noted in case of Ethanol extract of *P. Spinosa* extract. The similar trend was recorded in *P. Spinosa* extracts after 20, 30, 40 and 50 minutes.

DISCUSSION

It is known that the biological activity and nutritional value of any fruit depends not only on the amount of various biologically active and nutrients, but also on the component composition of these substances. Chromatographic (paper analyses) of the amount of sugars from prickly plum fruits showed the presence of 4 components in its composition. According to chromatographic data and comparison with authentic substances, their identity with glucose, fructose, xylose, sucrose was established. Of the simple sugars, fructose (2.82%), glucose (1.81%), sucrose (0.81%) and a small amount of xylose (0.18%) were found in the total sugars. Citric, malic and succinic acids were identified in organic acids by paper chromatography. Citric and malic acids make up the main part of organic acids, and succinic acid is found in an insignificant, trace amount.

The fruit contains 2.3% pectin. Pectin is useful in the prevention of various gastrointestinal diseases; it removes various toxic and radioactive elements from the body. Due to the high content of pectin, prickly plum fruits can be used in the confectionery industry. The qualitative composition and content of the nutritional components of prickly plum fruits make it possible to use it as a full-fledged fortified product to stabilize the defenses of the whole organism.

According to the content of vitamin C, prickly plum fruits surpass a number of fruit plants. For a person, the daily dose of vitamin C is 50-100 mg. From this amount of vitamin C (230 mg), it turns out that approximately 40 g of prickly plum fruits per day can fully meet a person's needs for vitamin C. Moreover, it is very important that vitamin C is combined in prickly plum fruits with a large amount of P vitamin-active anthocyanins, flavonoids which increases their digestibility.

Polyphenols, especially flavonoids, anthocyanins are of scientific and practical interest. Anthocyanins and flavonoids, along with vitamin P activity, also have antioxidant, anticarcinogenic, anti-inflammatory activity, are part of some medicines and harmless food dyes. As seen in the Table, the fruits of prickly plum are rich in anthocyanins (2.33%). By the method of two-dimensional chromatography, 3 anthocyanidins were found in the composition of the sum of anthocyanins. Two main components were isolated by preparative paper chromatography. According to the chromatography data, as well as the results of acid hydrolysis and their comparison with the literature data, the isolated components were identified as cyanidin-3-glucoside (chrysanthemin) and cyanidin-3,5-diglucoside (cyanine). The amount of flavonoids were isolated from the aqueous extract by extraction with ethyl acetate.

Chromatographic analysis of the sum of flavonoids revealed two spots by color in visible UV light and, when exposed to ammonia, gave characteristic colors for flavonoids. One aglycan was obtained by acid hydrolysis, which was identified as quercetin by chromatographic and spectral parameters. Two individual substances were isolated by preparative paper chromatography. The selected individual flavonoids after chromatographic, spectral analyzes, as well as acid hydrolysis and their comparison with the literature data were identified as quercitrin and rutin.

Numerous researchers have exploited the dietary analysis of *P. spinosa* fruits of various genotypes of Plum species, obtained from different localities. In our research work, values of dry matter were 29.31%. Many other researches showed dry matter values less than our findings plum (BY94M1945) with value of 11.1% (Cevallos-Casals *et al*., 2006) and Bluefre' with 12% dry matter (Walkowiak-Tomczak *et al*., 2008). Difference in dry matter content was perhaps owing to the dissimilarities in environmental and agronomic situations. Anthocyanin content is a highly important phytochemical for health of human beings and have been familiar as main causative complexes to antioxidant

activity in vivo and in vitro (Marchelak *et al*., 2017). Anthocyanin content in our research work was 2.33% corroborated by findings of Cevallos-Casals *et al*. (2006). Slight difference in value may be due to different agro-climatic situations.

Results of sugar contents in our research work were in line with findings of Melgarejo *et al*. (2012). Nisar *et al*. (2015) also reported pectin, organic acids, sugars and tannins contents in Plum species which corroborated our findings. Kristl *et al*. (2011) also reported the presence of phenolic contents in plum fruits which confirmed our findings. Results of TPC were confirmed by a study carried out by Erturk *et al*. (2009) and Sikora *et al*. (2013) on *P. spinosa* while Olawuyi *et al*. (2021) recorded TPC contents in *Pyrunus salicina*.

***Conclusion*:** It has been established that prickly plum fruits contain 29.31% dry matter, 5.44% total sugars, 4.01% organic acids, 2.31% pectin, 0.94% tannins, 0.32% flavonoid, 2 33% of the amount of anthocyanins and 0.23% of vitamin C. It is shown that the sum of sugars mainly consists of fructose (2.82%), glucose (1.81%), xylose is present in a trace amount and a small amount (0.81%) of sucrose. The sum of organic acids consists of citric, malic and succinic acids. The main ones are citric and malic acids. In the sum of anthocyanins, 3 components were found, of which 2 were isolated individually, and in the sum of flavonoids, 2 components were isolated. The isolated anthocyanins were identified as cyanidin-3-glucoside, cyanide-3,5-diglucoside, and flavonoids as quercitrin and rutin. Data on the quantitative and qualitative composition of biologically active and nutritional substances of fruits show that prickly plum fruits can be used as a promising raw material for the production of biologically active food concentrates and medicines.

***Author's contributions*:** T.Y. Abbasova and K. Sh. Dashdamirov conceived the idea, planned the study, and wrote the manuscript; H. Kh. Novruzova and F.U. Safarova did reviewing and editing; A.H. Asgarova and E.N. Novruzov assisted in the field work; in design layout and proof reading.

***Ethical statement*:** This is original research work and not yet published elsewhere and the authors declare that there is no conflict of interest.

***Conflict of interest*:** The authors declare that there is no conflict of interest.

***Consent to participate*:** All authors are participating in this research study.

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